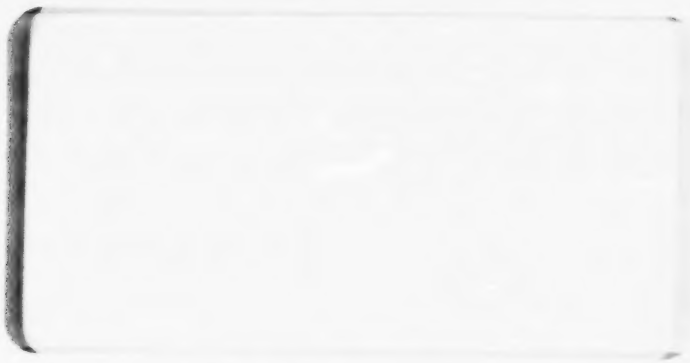

ADM

Acres Davy McKee

DSS File No.: 14SQ. 23440-6-9052
Serial No.: 23440-6-9052/01-SQ

Optimization of Fungal Adsorption of Uranium and Cell Immobilization and Pelletization

Final Report by:

Acres Davy McKee Engineering Inc.
Ontario Research Foundation
Denison Mines Ltd.
Dr. M. Tsezos

Work on the project was conducted under the auspices of the Canadian Centre for Mineral and Energy Technology, Energy, Mines and Resources Canada

DSS File No.: 14SQ.23440-6-9052
DSS Contract Serial No.: 23440-6-9052/01-SQ
CANMET Project No.:
CANMET Scientific Authority: Dr. R. McCready

64 pp

November 13, 1987

MICROMEDIA

ADM
Acres Davy McKee

November 13, 1987
C1065.00

Engineers and Managers

Dr. R. McCready
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Dear Dr. McCready:

Optimization of Fungal Adsorption of Uranium and Cell Immobilization and Pelletization
DSS File No. 14SQ.23440-6-9052
ADM C1065.00

Please find enclosed 3 copies of the Final Report for the above project.

We would like to thank you for the opportunity of working with you on this most interesting project.

Yours very truly,

David J. King

Dr. D.J. King, P.Eng.
Project Manager

DJK:saf
enclosure

cc: Mrs. D. Lamoureux (letter only)

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EXECUTIVE SUMMARY

In response to a request for a proposal from the Department of Supply and Services, a joint program was established by Acres Davy McKee Engineering Inc., the Ontario Research Foundation, Denison Mines Ltd. and Dr. Mario Tsezos to optimize the fungal adsorption of uranium and to determine a means of immobilizing the biomass.

A contract for the program was awarded to the consortium under DSS file no. 14SQ.23440-60-9052.

The program consisted of three stages

Stage 1 - Development of Detailed Work Plan
Stage 2 - Optimization of Nutrient Requirements and Fungal Adsorption of Uranium
Stage 3 - Preliminary Investigation of the Immobilization and Pelletization of the Biomass

Details of the program have been outlined in the proposal issued to DSS (ADM S3300.27) and are also discussed within the report.

The results of the study are summarized by the following conclusions:

(1) This program has demonstrated that it is possible to generate Penicillium sp. biomass with adsorption capacities in excess of 150 mg uranium/g dried biomass.

(2) The Medium TM is preferred for the production of biomass. Yields of 20-24 g dry weight/litre of Penicillium biomass can be obtained within 48-60 hours.

(3) The study has demonstrated that the Medium TM is best autoclaved in diluted form i.e., it is unstable when autoclaved as the concentrate. Decomposition products may be formed which may inhibit the initial growth of the Penicillium.

(4) Tests indicate that the optimum time to harvest the biomass is when an inflection is noted in the pH value of the culture medium.

(5) Allowing the mycelium to age while suspended in the culture medium (stationary phase of the growth cycle) did not afford significant increases in the uranium adsorption capacities of the biomass.

(6) Alternative industrial by-products, e.g. whey, may serve as potential substrates for the production of the biomass. The identification of the impact of the test protocol on the attained uranium loading on a test biomass suggests that the biomasses produced on carbon sources other than medium TM should be re-evaluated. The data may have important bearing on the economics of production of the biomass.

(7) Preliminary investigations indicate that the Penicillium biomass can be converted to immobilized and pelletized forms which retain high uranium adsorption capacities in excess of 150 mg uranium/g dried biomass.

RÉSUMÉ POUR LA DIRECTION

En réponse à une demande de proposition formulée par le Ministère des Approvisionnements et Services, un programme conjoint a été mis sur pied par Acres Davy McKee Engineering Inc., la Fondation de recherches de l'Ontario, Denison Mines Ltd. et le dr Mario Tsezos. Ce programme avait pour but d'optimiser l'absorption fongique de l'uranium et pour déterminer un moyen d'immobiliser la biomasse.

Un contrat pour ce programme a été décerné au consortium sous le nº de dossier DSS nº 14SQ.23440-6-9052.

Ce programme se sépare en trois stades

1er stade - Préparation d'un plan de travail détaillé

2e stade - Optimisation des besoins en éléments nutritifs et de l'absorption fongique de l'uranium

3e stade - L'investigation préliminaire de l'immobilisation et de la mise en boulettes de la biomasse.

Les détails du programme ont été soulignés dans la proposition émise au DSS (ADM S3300.27) et ont aussi été discutés dans le rapport.

Les résultats de l'étude sont résumés dans les conclusions suivantes:

1. Ce programme a démontré qu'il est possible de générer de la biomasse de <u>pénicillium</u> sp dont les capacités d'absorption de l'uranium dépassent 150 mg/g de biomasse sèche.

2. Le médium TM est préféré pour la production de biomasse. On peut atteindre un rendement de 20-24 g de poids sec/L de biomasse de pénicillium en 48-60 heures.

3. L'étude a démontré que le médium TM est mieux s'il est mis à l'autoclave sous une forme diluée, c.-à-d. qu'il est instable lorsqu'il est mis à l'autoclave en tant que concentré. Il peut se trouver des produits de décomposition qui empêcheront la croissance initiale de pénicillium.

4. Les essais ont montré que le moment optimal pour recueillir la biomasse se produit lorsque l'on remarque une inflexion de la valeur en pH du médium de culture.

5. Le fait de laisser le mycélium vieillir pendant qu'il est en suspension dans le médium de culture (phase stationnaire du cycle de croissance) n'apporte pas d'augmentations importantes dans les capacités d'absorption de l'uranium par la biomasse.

6. D'autres dérivés industriels, p. ex. le petit lait, peuvent servir de substrats possibles pour la production de la biomasse. L'identification de l'impact du protocole d'essai sur le chargement atteint d'uranium sur la biomasse d'essai semble indiquer que les biomasses produites sur des sources de carbone autre que le médium TM devraient être réévaluées. Cette donnée peut constituer un facteur important dans l'économie relative à la production de la biomasse.

7. Des enquêtes préliminaires ont indiqué que la biomasse de pénicillium peut être convertie en une forme immobilisée et en boulettes qui conserve des capacités élevées d'absorption de l'uranium, supérieures à 150 mg/g de biomasse sèche.

RECOMMENDATIONS FOR FURTHER WORK

The results from the program indicate that a number of areas exist which require further investigation beyond the scope of this project. In particular, the following elements should be addressed:

(1) Immobilized and pelletized Penicillium biomass be characterized in greater detail with regard to the adsorption/desorption isotherms.

(2) The stability of the immobilized and pelletized Penicillium biomass should be assessed under conditions of repetitive adsorption/desorption.

1. INTRODUCTION

Denison Mines, in co-operation with the Department of Energy, Mines and Resources, is conducting bioleaching of uranium underground. The volumes of leachate encountered in bioleaching are enormous and low in uranium concentrations. The costs of pumping millions of gallons per day of such leachate are very high and the Department wishes to determine the best means of producing and utilizing fungal biomass for the recovery of uranium underground.

The use of micro-organisms to recover uranium from Denison minewater has earlier been studied under DSS contract (15SQ.23440-5-9181) by Acres Davy McKee Engineering Inc. The adsorption rates, capacities, desorption characteristics, the effect of the pH and other cations on those parameters were studied in the aforementioned program. Amongst the treated micro-organisms, Penicillium provided superior adsorption and desorption characteristics. Uranium adsorption by Penicillium was however only about 25 mg/g of dry Penicillium.

The objectives of this present program are:

(1) Investigate the nutrient requirements to obtain a high (e.g. 200-250 mg U/g dry weight) uranium adsorptive capacity for inactivated Penicillium biomass.

(2) Investigate the ability of physical and chemical factors to alter the uranium adsorptive capacity of the fungal cell walls.

(3) Preliminary investigation of the immobilization and pelletization of the fungal biomass to allow its use in a packed column uranium recovery system.

The program should reflect the following important factors:

(a) A medium for commercial production of biomass should be inexpensive and use wherever possible industrial by-products. Thus, emphasis should be directed to evaluate industrial by-products as a carbon source.

(b) The yield of biomass per unit of fermenter capacity per hour will be important relative to the economics of producing a commercially viable product. Thus, although the highest possible uranium adsorption capacity is desired, if this criterion can only be met by cultural conditions affording low biomass productivity rates, the economics of the process will be suspect.

The details of the investigations for each of these areas are presented in the following report.

2. WORK PLAN

The work program consists of two phases with the first phase being split into two major areas. The original experimental program, as proposed, planned to assess the effect of a wide range of cultural parameters on uranium adsorption capacity using a limited number of shake flask tests. When it became apparent that the age of the biomass might be a critical factor, the work program was modified in consultation with the Scientific Authority to switch to experiments with stirred batch fermenters and obtain highly defined growth curves.

The modified work program incorporated the following:

Phase IA Investigation of nutrient requirements to obtain Penicillium biomass with a high uranium adsorptive capacity

The tasks include:

(a) establish basic protocols for culture vessels, culture conditions, harvesting procedure, inactivation procedure and uranium adsorption.

(b) cultivate biomass using the following carbon sources:

- Tsezos medium (TM)
- potato starch
- lactose
- maltodextrin
- cheese whey
- n-hexadecane

(c) carry out uranium adsorption tests on each of the above biomass samples.

(d) select an optimum medium based upon adsorption capacities for other phases of the program.

(e) submit an interim report.

Phase IB Investigation of the physical and chemical factors which may alter the uranium adsorptive capacity of the fungal cell walls

The tasks include:

(a) grow large scale cultures using the selected medium and investigate the following factors

- physiological age of biomass
- nutrient levels

(b) determine uranium adsorption capacity of representative samples of biomass from each test.

(c) select growth conditions for optimum uranium loading and biomass production per unit volume of fermenter.

(d) submit an interim report.

Phase II Preliminary investigation of the immobilization and pelletization of the fungal biomass

The tasks include:

(a) production of the biomass as per conditions selected in Phase 1.

(b) preparation of immobilized and pelletized fungal biomass

- M. Tsezos procedure
- ORF Sol Gel procedure

(c) performance of uranium adsorption test on each immobilized and pelletized biomass.

(d) evaluation of immobilized products.

(e) preparation and submission of final report.

3. MATERIALS & METHODS

3.1 Culture

The culture used in these tests was the acidophilic Penicillium sp., isolated by Dr. R.G.L. McCready, CANMET from the bacterial leach stopes at Denison Mines, Elliot Lake. Stock cultures were maintained on ager slants (0.1 strength nutrient broth + 1% (w/v) glucose, pH 2.3) at $4^{o}C$.

3.2 Basal Medium for Growth Studies

In order to undertake tests to assess the growth of the Penicillium sp. on different carbon sources, a basal medium was prepared consisting of:

1. Ammonium nitrate	20	g/L
2. Potassium dihydrogen phosphate	11	g/L
3. Magnesium sulphate (7 H_2O)	0.25	g/L
4. Zinc sulphate (7 H_2O)	0.03	g/L
5. Ferrous sulphate (7 H_2O)	0.05	g/L
6. Manganous sulphate (7 H_2O)	0.02	g/L
7. Ammonium molybdate (4 H_2O)	0.005	g/L

The medium was prepared as follows:

Solution A

The ammonium nitrate, potassium dihydrogen phosphate and magnesium sulphate were dissolved in distilled water and made up to 750 mL. The pH was adjusted to 5.0 with sulphuric acid prior to autoclaving at 16 psi for 25 minutes.

Solution B

A 100X concentrated solution of zinc sulphate, manganous sulphate and ammonium molybdate was prepared with distilled water, and the pH was adjusted to 5.0 with sulphuric acid prior to sterilization by autoclaving at 16 psi for 25 minutes.

Solution C

A 100X concentrated solution of ferrous sulphate was prepared in distilled water, pH 5.0, and sterilized by filtration (0.22 μm pore size filter) immediately before use.

The basal salts medium was prepared for tests by aseptically mixing the appropriate volumes of Solutions A, B, and C.

The carbon source being evaluated was dissolved in the appropriate volume of water and sterilized separately at 16 psi for 20 minutes. It was added to the the basal salts solution and, if necessary, sterile distilled water was added so that the final volume of the combined medium was one litre. In some instances with the preparation of culture media containing substrates such as starch, the concentrated solution of the carbon source might be too gelatinous to disperse properly in the basal salts solution. In such cases, the basal salts solution was prepared at double strength (2X concentrated) so that the medium with the highest carbon content could be prepared by aseptically mixing equal volumes of the sterile basal salts solution and the sterile solution of the carbon source. Thus, the latter solution could be prepared as a more dilute, less viscous solution.

3.3 Preparation of Culture Media from TM Concentrate

Medium TM is a proprietary medium developed by Dr. Tsezos and was supplied as a sterile concentrate for preparing various strengths of media. Several protocols were used throughout the experimental program.

The concentrate as received was dark brown in colour and it was stored (800 mL volumes) at $4^{o}C$ until use. To prepare the medium as recommended by Dr. Tsezos (i.e. hereafter referred to as medium TM), this concentrate (2 volumes) was diluted with water to 5 volumes.

In initial experiments, this dilution was carried out by aseptic transfer to sterile shake flasks or fermenters with the addition of sterile water. Concern about the tendency of low numbers of contaminant organisms to appear in inoculated cultures prior to the growth of the Penicillium inoculum, led to diluting the concentrate and resterilizing it in situ in the fermenter and/or culture vessel. For preparation of 5L of medium in a 7.5L fermenter, diluted medium was autoclaved for one hour at 16 psi.

3.3.2 Media Based on Dilution of Non-Sterile TM Concentrate

In later stages of the program, the TM concentrate was supplied as a non-sterile preparation which was stored at -10°C. The concentrate was thawed as required and diluted with distilled water prior to addition to a fermenter (5L operating volume). The medium was autoclaved in the fermenter at 16 psi for one hour. The non-sterile concentrate was golden brown in colour. Media TM prepared as described in the laboratory retained the gold/brown colour.

3.4 Culture Conditions

3.4.1 Shake Flask Cultures

Shake flask cultures were conducted in 500 mL baffled culture flasks containing 150 mL of culture media. Inoculation was with 7.5mL of an actively growing culture of the Penicillium sp. and cultures were incubated at room temperature (23-25°C) with shaking at ~160 rpm (New Brunswick Gyrotory Shaker, Model S-3).

3.4.2 Large Scale Tests-Stirred Tank Fermenters

Large scale cultures were grown in 7.5L laboratory fermenters (New Brunswick Microferm). The operating volume was typically 5 L of medium with an inoculum of 300 mL of an actively growing culture of the

Penicillium sp. The fermenters were equipped with three multiple blade impellers and four vertical baffles. Sterile air was introduced via a single orifice sparger situated beneath the lower impeller. All cultures were incubated at 23°C. Intermittent cooling of the culture broth was required to maintain the temperature below 25°C. This was particularly noticeable when high agitation rates were used with broths containing high biomass densities. Cultures were sampled at appropriate intervals by aseptically withdrawing an aliquot of the broth via a sampling line extending to the bottom of the fermenter.

3.5 Harvesting Procedures

Biomass was harvested by filtration of a sample of culture broth through a preweighed pad of cheesecloth. The biomass was then washed exhaustively with warm tap water *i.e.* until only slight colour was present in the filtrate, when the cheesecloth and biomass was squeezed gently to expel entrained water.

In order to monitor biomass production in cultures, the filter and sample were dried *in vacuo* at 40°C and then weighed to determine the biomass weight. In shake flask tests, the total content of a flask would be harvested. In fermenter cultures, a suitable volume was taken (50 - 100 mL depending on the biomass concentration).

A number of protocols were investigated for the harvesting and inactivation of biomass prior to conducting the uranium adsorption tests. The preferred procedure used throughout the program involved:

(a) collection of the biomass by filtration through cheesecloth
(b) washing of the biomass with warm tap water until the eluate was relatively free of colour
(c) suspension of the biomass in warm tap water for 30 minutes

(d) refiltration on to cheesecloth
(e) repetition of the warm water wash
(f) inactivation of the biomass by autoclaving (16 psi for 20 minutes)
(g) freeze-drying.

The dried biomass was stored in sealed bottles in dessicators until used.

3.6 Tests for Biomass Uranium Adsorption Capacity from Denison Mines Water

3.6.1 Test Solutions - Mine Water

The mine water as received from Denison Mines Ltd. had a pH of 2.05 and contained approximately 180 ppm U. For the adsorption testing, the pH was raised to 4.0 with saturated sodium hydroxide solution. Raising the pH to 4.0 cause precipitation of iron in the mine water. The precipitate was removed through settling and decantation. The pH adjusted mine water was filtered (0.45 micron pore size) prior to adsorption testing.

Two multielement analyses were performed on pH adjusted mine water. The results are shown in Table 1.

For an adsorption test with Biomass P2-12, a synthetic uranium solution was made up from U_3O_8 (yellow cake) dissolved in sulphuric acid. The pH was adjusted to 4.0 with sodium hydroxide solution. The U content was 193 ppm (cf. Tests 87-11, -12, -13).

3.6.2 Adsorption Test Protocols

Adsorption tests were conducted in 500 mL Erlenmyer flasks. A Burrell wrist action shaker was employed to agitate the test suspensions throughout the contact period at a rate of (approximately) 300 oscillations per minutes.

Freeze-dried biomass test samples were weighed on an analytical balance. If residual moisture was suspected to be present in the freeze-dried materials, triplicate moisture determinations were made. The weight of dried biomass solids in a test was calculated from the moisture content.

The preliminary testing of biomasses P1 and P2 was conducted with 100 mL volumes of mine water (Tables 2 - 4). However, the subsequent loading isotherm testing for biomasses P2 - P10 was based on 200 mL samples of mine water per test. The remaining adsorption tests were with 400 mL samples of mine water (Biomasses P11 - P12).

With the exception of tests with biomass P1, which was in contact with the mine water for 90 hours, the contact time was 24 hours throughout the adsorption capacity testing.

4. EXPERIMENTAL PROGRAM

The experimental program consisted of three phases:

4.1 Selection of the optimum growth medium

4.2 Investigation of the effect of biomass age on uranium adsorption capacity

4.3 Production of biomass and investigation of procedures for the immobilization and pelletization of the biomass.

The following description of the experimental program identifies the major emphasis in any experiment according to the experimental category in which it is included. However, some overlap will be noted. For example, the observations that the age of the biomass might be a significant factor led to the age factor being included in the assessment of candidate media.

4.1 Selection of Optimum Growth Medium

The objective of this stage of the experimental program was to select the medium which afforded Penicillium biomass with the optimum capacity to absorb uranium from the Denison leach liquor. Factors of economic significance would be the biomass yield per unit volume of fermenter capacity as well as the cost of the ingredients. Therefore, industrial wastes appeared to be potential low cost feedstocks.

Previous studies had indicated that Penicillium biomass cultured on low molecular weight sugars tended to have low uranium adsorption capacities and since substrate availability will influence the rate of growth and the chemical composition of the mycelium, the effect of using refractory carbon sources would be investigated, e.g. Medium TM, hexadecane, etc.

4.1.1 Experiment PE 1. Preliminary Shake Flask Tests - Medium TM

In a preliminary screening test using baffled shake flasks, the sterile TM concentrate was diluted X2.5, X5, X10 and X25 with the basal salts medium. The X2.5 dilution of the concentrate afforded the Medium TM. Therefore, the other dilutions gave media which were 1/8th strength (Medium TM/8), 1/4 strength (Medium TM/4) and 1/2 strength (Medium TM/2) respectively. Cultures, (150 mL in 500 mL baffled culture flasks) were each inoculated with 7.5mL (5% v/v) of a 48 hour culture of the

acidophilic Penicillium sp. in basal salts/glucose medium. The cultures were then incubated with shaking (160 rpm) at room temperature (23°C). Good growth was noted in each strength of the culture medium and biomass concentrations were determined at various selected time intervals. However, there were insufficient data points to establish growth curves and problems were encountered due to the presence of suspended inorganic material in the medium.

4.1.2 Experiment PE 2. Repeat of the Shake Flask Tests - Medium TM

The experiment described above was repeated with a modified TM concentrate which did not contain added suspended particulates. This modified media was used hereafter in the study. In this case, the concentrate was diluted with sterile distilled water to give the Medium TM. The more dilute media were prepared by adding the same proportion of sterile distilled water to the concentrate with the balance being made up with the basal salts medium. Four flasks were inoculated per medium using a 3% (v/v) inoculum of the Penicillium sp. Incubation conditions were as described above. Biomass from each culture flask was recovered by filtration through a preweighed pad of cheesecloth. The biomass on the cheesecloth was washed well with distilled water and gently squeezed to expel as much as possible of the residual constituents of the medium (oils, etc.). The data for the biomass concentrations expressed as gram dry weight per litre (g.d.w./L) are plotted versus time (Figure 1).

4.1.3 Experiment P 1. Preliminary Culture in Stirred Tank Fermenter - Medium TM

These tests used Medium TM (i.e. sterile concentrate diluted x 2.5 with sterile water). The cultures were carried out in 7.5L Microferm Fermenters (New Brunswick Scientific) with an operating volume of 5.5L. The medium, inoculated with 300 mL of an actively growing culture of the

Penicillium sp., was incubated at room temperature with mechanical stirring (400 rpm) and an aeration rate of 5 L/min. As the biomass density increased, the broth suspension became very viscous and the agitation was increased to 600 rpm.

4.1.4 Experiment P 2. Repeat of the Growth Curve in Stirred Tank Fermenter - Medium TM

Experiment P 1 was repeated with the following modifications. Larger baffles were installed on the stirrer shaft and positioning of the baffles (height in the liquid) was adjusted so as to minimize the tendency for biomass to spray to the upper glass surfaces. The Medium TM, prepared by dilution from the concentrate (although sterile when received), was further autoclaved to ensure sterility.

The initial culture conditions were agitation at 400 rpm and aeration at 6 L/min. By the end of the test (200 h), the agitation had been increased to 500 rpm. Biomass samples were recovered at various time intervals. The data for the biomass concentration (g.d.w./L) and the pH of the broth are plotted against time (Figure 2).

Samples of the biomass were collected on cheesecloth at 82.5, 147, and 200 h incubation periods. The biomasses were washed extensively with tap water and the 200 h sample was suspended in tap water for 48 h at 4^{o}C. The entire samples collected at 82.5 and 147 h and a portion of the sample at 200 h were autoclaved at 16 psi for 20 min. to kill the biomass. Aliquots of the biomass at 200 h were also "inactivated" by either boiling in 80% ethanol (75^{o}C) for 15 min. or heating at 80-85^{o}C in tap water for 15 minutes. The biomasses from all inactivation tests were washed with sterile distilled water to remove any organics released during the heat treatment. The biomasses were then recovered on cheesecloth and freeze-dried for storage and determination of the uranium adsorption capacity.

4.1.5 Experiment PE 3. Growth of *Penicillium* sp on Alternative Carbon Substrates - Preliminary Shake Flask Cultures

Preliminary tests were carried out in baffled shake flasks to assess the ability of the organism to utilize other carbon sources. Those investigated were lactose, maltodextrins, potato starch and n-hexadecane. Positive growth was obtained in culture media containing each carbon source in the basal salts medium (pH 5). Dense fibrous/pelletized growth was obtained with lactose, maltodextrins and potato starch. The biomass with n-hexadecane as substrate tended to be much larger pellets and developed very strong orange pigmentation whereas with the other substrates, the biomass was creamy beige in colour.

Growth curves were not done at this time. However, samples of the biomass were cultivated in shake flasks and harvested after approximately 120 h. The pH values of the broths at the time of harvest were potato starch (~5.2), lactose (~3.2) and maltodextrin (~4.0). The washed biomasses were inactivated by autoclaving and freeze-dried. Uranium adsorption tests were carried out.

4.1.6 Experiment P 3. Repeat of Experiment P 2. - Medium TM

This was a repeat of Experiment P 2 except that a lower aeration and agitation rate was used (*i.e.* 400 rpm at 3 L/min. for P 3 compared with 400 - 500 rpm at 6 L/min. for P 2). Biomass was monitored throughout the growth curve (Figure 3) in order to identify the amount of storage compounds in the fungal cells. Biomass was harvested when extensive vacuolation was noted. Filtered and washed biomass was suspended in tap water for 48 hr. at 4^{o}C prior to autoclaving and freeze-drying. The biomass was assessed for uranium uptake.

4.1.7 Experiment P 4. - Growth Curve in Stirred Tank Fermenter - Medium TM/4

This culture used Medium TM/4. Therefore, more rapid depletion of carbon source was expected. The growth of the biomass was monitored (Figure 4) and at the appropriate time interval, it was harvested using a similar protocol as for P 3.

4.1.8 Experiment PE 6. Alternative Carbon Substrates - Shake Flask Tests

Culture media were prepared in the basal salts solution with starch, lactose and maltodextrin as substrates (5% w/v). Cultures (150 mL) were inoculated with 7.5 mL of an actively growing culture of the Penicillium sp. and incubated at 23°C with shaking (~160 rpm). Cultures were harvested after 5 days at which time there was dense growth in the cultures with starch, lactose and maltodextrin. The biomasses from the starch medium (Culture PE6-1), the lactose medium (Culture PE6-2) and the maltodextrins medium (Culture PE6-3) were each autoclaved and freeze-dried. Uranium adsorption capacities were determined.

4.1.9 Experiment P 5. n-Hexadecane Medium - Stirred Tank Fermenter

Experiment P 5. was carried out at 23°C in a stirred tank fermenter using n-hexadecane (5% w/v) plus 0.1% (w/v) Tween 80 in basal salts medium. Foaming was a major problem and it was necessary to maintain the aeration rate at a lower than desired level (~1L/min). Growth of the Penicillium was negligible and after ten days' incubation the experiment was terminated. The very small amount of biomass was recovered and an adsorption test was performed.

4.1.10 Experiment P 6. Cheese Whey - Stirred Tank Fermenter

An aliquot (2.25 L) of sweet cheese whey (30% solids ≡ ~ 10% w/v lactose)) was diluted with an equal volume of basal salts solution (2X concentrated). The mixture was transferred to a Microferm fermenter and autoclaved in situ - 16 psi for 30 minutes. The cooled medium containing a significant amount of suspended solids was inoculated with 500 mL of an actively growing culture of the Penicillium sp. The pH of the culture medium was 5.3. The culture was agitated at 400 rpm (stirrers equipped with only two impellers) and aerated at ~ 1 L/minute. The lower aeration rate was also necessary to reduce foam generation. Foam was controlled by the manual addition of sterile antifoam solution (1% w/v Dow Corning 1520) as required. A growth curve was prepared (Figure 5). Samples of biomass were collected at selected intervals, and washed and freeze-dried as per the usual protocol.

4.1.11 Experiment P 7. Cheese Whey - Stirred Tank Fermenter

This was a repeat of Experiment P6. However, in this instance the volume of whey medium in the fermenter was reduced to 4 litres in order to attempt to control foaming. This was a severe problem with this culture medium and it was necessary to add significant quantities of sterile antifoam solution (Dow Corning 1520). Agitation at 400 rpm was acceptable but the aeration rate was maintained at ~ 1 L/min. to attempt to control the foaming. Following foamover of the culture at Day 5, contaminants were noted in the culture which was then terminated.

4.2 Effect of Biomass Age on Uranium Adsorption Capacity -

4.2.1 Experiment P 8. Medium TM/2 - Stirred Tank Fermenter

Medium TM/2 was prepared by diluting the concentrate five fold (5X). Sterilized medium (5 L) was inoculated with 450 mL of an actively growing culture of the Penicillium sp. in glucose/basal salts media. The culture was incubated at room temperature with an agitation rate of 400 rpm and aeration set at 4L/min. A growth curve was established and the morphology of the fungal biomass was examined microscopically throughout the experiment. Samples were taken and prepared for uranium adsorption tests at selected intervals along the stationary phase of the growth cycle (Figure 6).

4.2.2 Experiment P 9. Medium TM/1.5

This experiment was carried out in identical fashion to Experiment P 8 except that the Medium TM/1.5 was prepared by diluting the concentrate x 3.75. As before, the growth of the organism was monitored (Figure 7) and samples taken at appropriate intervals for uranium adsorption tests.

4.2.3 Experiment P 10 - Medium TM - Stirred Tank Fermenter

This was conducted in similar manner to the previous cultures except that the media was the regular strength Medium TM. The sampling protocol was designed such that samples (400 - 500 ml) of the culture broth were harvested at 24 hour intervals from the start of the stationary phase of the growth cycle, i.e. when the pH began to rise sharply (Figure 8). As the volume of the broth was lowered with successive samplings, biomass was sprayed heavily on to the surfaces of the fermenter above the liquid. The biomass samples were washed and freeze-dried for uranium adsorption tests. However, results are subject to question due to the experimental difficulties.

4.2.4 Experiment P 11 - Medium TM - New Preparation Procedure - Stirred Tank Fermenter

This was the first experiment using the new preparation procedure for the medium. In this instance, non-sterile frozen concentrate was diluted as per normal (2 volumes → 5 volumes) with distilled water and transferred to the 7.5L fermenter. The medium was autoclaved in the fermenter for 1 hour at 16 psi. The cooled medium was restored to volume (4.7L) and inoculated with an actively growing culture of the Penicillium sp.(280 mL). The culture was incubated at 26°C. with agitation (200 rpm) and aeration (3L/min). As the biomass density increased (16 h), the agitation rate was increased to 400 rpm. A growth curve was constructed (Figure 9) and the total culture was harvested as soon as a rise in pH was noted.

The harvested biomass was washed extensively by the prescribed protocol. The total biomass was then autoclaved (16 psi, 30 minutes) to inactivate it prior to freeze-drying. The freeze-dried biomass was used for characterization of the adsorption process and immobilization/pelletization studies.

4.2.6 Experiment P 12. Medium TM - Stirred Tank Fermenter - Repeat of Experiment P 11

The experiment was a repeat of Experiment P 11 except that only the pH of the culture was monitored. The biomass was again harvested when the pH rise (Figure 10) indicated that the growth curve should be approaching the stationary phase. The harvested biomass, washed free of medium constituents, was then freeze- dried. It was then characterized for uranium adsorption prior to undertaking immobilization studies/pelletization .

4.2.7 Experiment P 13. Extended Growth Curve with Medium TM - New Preparation Procedure - Stirred Tank Fermenter

The large-scale preparation of biomass (Experiments P 12 and P 13) used Medium TM which received only a single autoclaving after dilution. As indicated in the growth curves (Figures 9 and 10) no significant lag phase was noted. Since the medium was now more favourable for growth of the Penicillium sp., a more complete growth curve was determined. Culture P 13 was a repetition of Cultures P 11 and P 12 except that upon reaching the stationary phase of growth, the culture was monitored for an additional 5 days of incubation (Figure 11).

4.2.8 Experiment PE 8. Investigation of Effect of Medium Sterilization Protocol on Growth of Penicillium sp - Shake Flask Culture

This experiment was conducted in shake flasks containing Medium TM which had each been sterilized only once by autoclaving as either the concentrate or the regular strength. The latter medium was autoclaved in the baffled shake flasks. The former medium was prepared by aseptically transferring the autoclaved concentrate to a sterile flask and diluting it to volume with sterile distilled water. The medium was then dispensed aseptically into the shake flasks.

The cultures, each inoculated with Penicillium starter culture (10 mL), were incubated at 23^{o}C with shaking (180 rpm). At selected time intervals based on the visual observations of fungal growth, the total contents of a culture were harvested to determine the biomass yield. The respective growth curve for each medium was plotted. (Figure 12)

4.3 Biomass Immobilization

Freeze-dried biomass prepared in Experiment P11 and P12 was used in the immobilization studies. Two separate immobilization procedures were investigated - one at Dr. Tsezos laboratory, and the other at Ontario

Research. Both procedures are proprietary with patent applications either pending or being submitted. Therefore, the specific details of the preparation procedures cannot be disclosed at the present time.

The immobilized biomasses (in the form of pellets) were assessed for their respective uranium adsorption capacities and their individual kinetics of the adsorption/desorption process.

5. DISCUSSION OF RESULTS

The results of the experiments described previously are shown in Tables 1-14 and Figures 1-12.

Throughout the experimental program, biomass samples were collected for adsorption testing at selected intervals along the growth curve in an experiment. In the tables, the sample identification includes the Experiment No. (e.g. P2) followed by a number identifying the point on the growth that the biomass was sampled (e.g. P2-12). Where feasible, the location of this sampling point is shown on the respective figure containing the growth curve.

In the figures, the individual data points have been connected. However, in all instances, the trend of the growth curve is the important characteristic.

5.1 Selection of Optimum Growth Medium

5.1.1 Selection of Culture Conditions

As outlined in the introduction, the experimental protocols used in the major portion of this program were based on large-scale cultures in 7.5 L stirred tank fermenters. This change from the proposed experimental protocols was taken, following discussion with the Scientific Authority, in response to the information obtained from preliminary experiments in shake flask cultures.

The preliminary cultures in shake flasks using Medium TM indicated that there was an apparent decline in the yield of biomass per unit volume of culture suspension as the culture aged (Figure 1). This was expected, but it was apparent that more frequent monitoring of cultures was required in order to establish accurate growth curves.

It was also observed in the shake flasks that the medium strength influenced the type of mycelial growth. Thus, in the more dilute media (Media TM/4 and TM/2), the biomass consisted of larger pellets. Since fibrous growth was preferred for the immobilization procedures, this pelletized growth was not desired. By using larger scale cultures in stirred tank fermenters, the aeration and mechanical agitation could be selected to discourage the formation of pellets.

5.1.2 Comparison of Carbon Sources

Based on the preliminary shake flask tests, it was apparent that high yields of biomass could be obtained with Medium TM and that the maximum biomass yield per unit volume of culture was proportional to the carbon concentration of the respective strengths of medium (Table 1). Thus, other nutrients were not limiting.

In an earlier study, biomass cultured in a medium containing predominantly glucose as a carbon source had low uranium adsorption capacity. It was postulated that this reflected an excess production of reserve storage compounds in the presence of an easily metabolized energy source. Accordingly, an objective of this study was to investigate carbon sources which were more refractory and hency more slowly utilized by the organism.

An objective of the study was to assess the potential use of industrial products as media constituents. Penicillium sp. was cultured in both shake flasks and larger scale cultures to assess the effect of other potential

carbon sources on the uranium adsorption capacity of the biomass. Technical grade substrates were used, e.g. lactose, maltodextrins, potato starch and n-hexadecane, as representatives of key carbon sources in potential industrial by-products, e.g. whey, distillery waste, potato waste, oils, etc.

With the exception of n-hexadecane, good growth was obtained with all carbon sources. Slight growth obtained with n-hexadecane in shake flask tests was not duplicated in the larger scale culture. It is believed that the growth in the shake flask may have been due to growth of the Penicillium on residual carbon nutrients introduced with the inoculum. In the large scale culture, the starter inoculum was cultured in shake flasks using the hexadecane as carbon source, hence an easily metabolized carbon source was not available. Negligible fungal growth was observed.

The good growth obtained with lactose as carbon source prompted the assessment of sweet whey medium. In all large scale cultures, foaming was a constant problem. In the case of the whey, it proved very difficult to control and required the addition of excessive amounts of sterile silicone antifoam solution. Good yields of biomass were obtained but the uranium adsorption capacities proved to be poorer than those of biomass cultured on Medium TM.

The data indicated that no benefits were derived from the use of substrates/media other than the Medium TM. Accordingly, Medium TM was selected for more intensive investigation.

5.1.3 Medium TM

A general analysis of TM concentrate is as follows:

Total Solids	- 28% (w/v)
Total Phosphorus	- 1.1% (w/v)
Magnesium	- 0.5% (w/v)
Total Kjeldhal Nitrogen	- 11% (w/v)
Total Organic Carbon	- 11% (w/v)

The media prepared by dilution of the sterile concentrate were dark brown in colour. An inorganic particulate was included in an initial batch of the product but this was excluded for subsequent experiments.

Preliminary shake flask tests with Medium TM (Figure 1) indicated that it supported very good growth of the Penicillium; the yield was greater than 17 g dry biomass per litre. However, during the course of the experimental studies on Medium TM, a pattern was identified which suggested that medium was sensitive to autoclaving. The initial experiment (Figure 1) was conducted with concentrate which, received sterile, was only diluted with sterile water. An initial large-scale test (Figure 2) was also conducted on the medium prepared in this manner. However, as noted in the figures, a significant lag phase was noted with those cultures, i.e. 40 - 70 hours. This lag phase was longer than expected from other fungal growth studies.

In order to prevent the development of contaminant organisms in the medium prior to the initiation of the fungal growth, the Medium TM prepared by dilution of sterile concentrate was also autoclaved in situ in the fermenters. This additional autoclaving of the medium did not change significantly the length of lag phase in test cultures.

As studies were completed with various dilutions of TM concentrate, a possible relationship was noted between the length of the lag phase and the concentration of the medium (Figures 4, 6, 7, and 8; Table 3A).

A test was performed in which Medium TM (prepared from sterile TM concentrate) was autoclaved twice prior to inoculation. The growth of the Penicillium was almost totally inhibited in this medium. Thus, it appeared that the autoclaving was giving rise to either toxic degradation products or to the destruction of essential nutrients/co-factors in the medium.

A modified media preparation procedure was instituted whereby non-sterile concentrate was diluted prior to sterilization *in situ* in the fermenters. Inoculated cultures showed immediate and rapid growth of the *Penicillium*, with active growth completed within 40-48 hours (Figures 9 and 10). The medium prepared in this fashion was much lighter in colour after autoclaving than the corresponding Medium TM prepared from sterile concentrate. These observations were confirmed in the more extensive growth curve (Figure 11). In parallel shake flask culture tests, the growth of the *Penicillium* was compared in Medium TM prepared from sterile concentrate and Medium TM sterilized by autoclaving after dilution of non-sterile concentrate.

Thus, growth of the *Penicillium* was almost completed within 40 hours in Medium TM sterilized after dilution. At that incubation period, the growth of the *Penicillium* was only beginning in the Medium TM prepared from sterile TM concentrate.

It is apparent that the Medium TM cannot withstand autoclaving in a concentrated form. This approach was instituted to prevent growth of contaminant organisms during shipment between laboratories. However, keeping the concentrate frozen until use and exposing it to a single sterilization by autoclaving prevented the inhibition of growth of the *Penicillium* sp.

In the shake flask test (Figure 12), a higher biomass yield was noted in the Medium TM prepared from sterile concentrate. This may indicate that the autoclaving of the medium in concentrated form not only gave rise to toxic degradation products but may also cause partial breakdown of the refractory carbon source making it more available for growth of the organism. This observation was not substantiated by the limited number of large- scale cultures using the Medium TM sterilized in diluted form. From the economic standpoint, the long lag phase would be more significant due to the reduced productivity (g dry biomass/L/hour) from the fermenter capacity.

TABLE 3A - RELATIONSHIP BETWEEN THE LENGTH OF THE LAG PHASE FOR GROWTH OF PENICILLIUM sp. IN MEDIUM TM AND MEDIUM TM CONCENTRATION

Medium	Dilution Factor Relative to TM concentrate	Length of Lag Phase (hours)
Medium TM/4	x 10	Negligible
Medium TM/2	x 5	Negligible
Medium TM/1.5	x 3.75	72
Medium TM	x 2.5	70 - 80

5.2 Effect of Age on the Uranium Adsorption Capacity of Biomass Cultured in Medium TM

Tests to assess the effect of age of the Penicillium biomass on its uranium adsorption capacity were concerned primarily with whether or not the lower than expected uranium loadings were related to the presence of non-adsorbing storage compounds in the fungal cells. Thus, at the end of the linear growth phase, the biomass yields in many of the media investigated were high but the cells, when examined by phase contract microscopy, were filled with granular storage materials. While the cells were maintained in the "stationary phase" of the growth cycle, a decrease in biomass concentration/unit volume of culture was noted together with the development of extensive vacuolation in the cells. Thus, it was believed that by maintaining the cells under aeration and agitation, it might be possible to cause the cells to deplete their reserve of energy compounds.

In the current study, assessing the relative age of the biomass was complicated by the extended lag times noted for the growth of the mycelia in test media. The length of the lag period also varied widely between cultures with different concentrations or composition of medium. Therefore, for this study, the reported age of biomass was calculated in each case from the mid-point of the linear phase of the respective growth cycle.

5.2.1 Preliminary Tests

Biomass harvested from the initial large scale culture (P1) afforded relatively poor uptake of uranium (33.3 mg U/g biomass) from the mine water which had been adjusted to pH 4.0 (Table 2). However, this biomass was relatively young (55 hours into the stationary phase) and the low uptake was attributed to the presence of excess storage compounds. When this experiment was repeated (Experiment P2), significantly better uranium loadings were noted (Table 3) with loadings reflecting both uranium concentration in the test solution as well as the biomass concentration in the test. The higher loadings were obtained with a synthetic uranium solution (193 ppm U) but the test could not differentiate whether the reduced loading with the mine water solution is a reflection of either the lower uranium concentration or the presence of compounds in the mine water which interfere in the adsorption of uranium by the biomass. However, the mine water sample used in this test had been stored for over one month at the elevated pH (4.0) and it was possible that changes had occurred in the chemical speciation of the uranium in solution.

5.2.2 Inactivation Procedure

In the series of tests for Experiment P2, no significant benefit was observed when the biomass was inactivated by either boiling in ethanol or heating at 85°C as an aqueous suspension. Accordingly, throughout the remainder of the test program, washed biomass was inactivated by autoclaving at 16 psi for 20 minutes prior to being freeze-dried for storage.

5.2.3 Impact of Test Protocol on Uranium Adsorption

The data from Experiment P2 also indicated that the maximum loading capacity was influenced significantly by the ratio of the biomass solids (g) to solution volume (mL). Accordingly, for subsequent tests, the ratio was decreased typically to 0.1 g dried biomass/200 mL minewater instead of the 0.1-0.5 g/100 mL ratio used in Expt. P2.

It was noted that when the ratio was further decreased to 0.01 g dried biomass/200 mL (Tables 6 and 7), an equivalent improvement was noted in the observed adsorption capacities. A similar effect was noted in Tables 11-13 when tests were performed at biomass/solution ratios of 0.02 g dry weight/400 mL mine water.

These data advise caution in deciding the maximum adsorption capacity that was attained with these biomass samples. Because of the small weights of biomass used in the tests, small differences in the uranium concentration of a supernatant solution can significantly change the calculated loading. Thus, the inherent limits on the precision of the D.C. Plasma Atomic Emission analytical procedure will reduce the overall precision of the loading values.

5.2.4. Effect of Biomass Age on Adsorption Capacity

In the course of this study, inactivated, freeze-dried biomass samples of varying ages were assessed for their respective capacities to adsorb uranium from the Denison mine water. At times, a positive correlation appeared to be noted between age and uranium uptake from solution, but subsequent experiments failed to confirm those preliminary observations.

Thus, the results of tests with biomass P2-8 (42.5 hours) and P2-11 (105 hours), when compared with the data for P2-12 (159 hours), suggested that aging the biomass did result in a slight improvement in the relative loading capacity.

On testing aged biomass samples recovered from cultures P3-13 (210 hours) and P4-11 (270 hours), the data indicated that no significant enhancement was noted for the uranium uptake capacity as a result of the aging process. Similarly, biomass P4, cultured in Medium TM/4, showed only a slight improvement in the uranium loading compared with biomass produced using the regular strength medium.

These tests were also conducted with the aged mine water solution and when a fresh sample was forwarded to ORF by Denison Mines, the adsorption tests with biomasses from cultures P2, P3, and P4 were repeated (Table 6 and 7). The uranium concentration of this mine water was 135 ppm compared with 66-92 ppm concentrations noted in the older water sample. The variations in the uranium concentration with age suggested that the uranium in solution was unstable and most probably being lost when the solution was filtered prior to each adsorption test.

Data obtained with the new mine water sample, with the pH adjusted immediately prior to an adsorption test, indicate significantly higher uranium loadings for biomasses P2-12 with the maximum being obtained in the test mixture with the highest ratio of liquids/solids (i.e. Test 87-41, Table 6).

Biomass P3-13 (210 hours) also showed a significant improvement over the previous set of tests (cf. Tables 5 and 7), but no change was noted for the uranium loading of biomass P4-11 (Table 7).

An initial series of tests with biomass harvested from Medium TM/2 (Culture P8) and Medium TM/1.5 (Culture P9) afforded poor uranium loadings (Table 8). This was attributed to the recurring problem with the stability of the uranium in mine water which had been adjusted to pH 4.0 (cf. the lower uranium concentration in the feed solution). When the tests were repeated with a freshly adjusted sample of mine water (uranium concentration 150-153 ppm), the uranium loadings were improved (Table 9). As noted previously, increasing the liquid/solids ratio in the test increased the observed uranium loading for sample P8-12A. However, comparison of the uranium loadings obtained under equivalent test conditions (0.5 g dry biomass/200 mL mine water) with biomasses sampled at intervals along the stationary phase of growth in both cultures indicate that the age of the biomass had only a slight impact on the observed uranium loading.

Thus, biomass P8-15A (239 hours) was more effective than biomass P8-12A (129 hours), while sample P9-13A (180 hours) gave a slightly higher loading than P9-11A (85 hours). However, these benefits were outweighed by the impracticality of maintaining cultures in the stationary phase of the growth cycle for such extended periods (8 - 10 days). Of particular note is the extremely poor loadings observed with samples aged for an extensive period (12 - 14 days; P8-Final and P9-Final). At the time of sampling, these cultures were both showing a renewed increase in the respective biomass concentrations per unit volume of culture. This was attributed to secondary growth of the mycelia occurring on by-products of their own autolysis during the extended holding time in the stationary phase.

The test cultures with diluted concentrations of Medium TM showed no benefits for the uranium adsorption capacities of the biomass samples generated in those media when compared with biomass produced in the higher yielding regular strength medium. A final test was performed in which larger biomass samples were harvested at 24 hour intervals from a culture of the Penicillium in the regular strength TM medium. As the volume of the culture was reduced by the sampling (500 mL samples from an initial volume of 5.5 L), problems were encountered due to spraying of the biomass on to the surfaces of the fermenter above the level of the culture broth and an unexpected sharp decline was noted with time in the biomass concentrations per unit volume of culture (Figure 8). The tests of the uranium adsorption capacities of the biomass samples harvested at 24 h intervals along the stationary growth phase of Culture P10 failed to show any improvement in the uranium loadings with age (Table 10). Because of the experimental problems in the fermenter, the test data may not be representative.

Based on the complete range of tests conducted in different strengths of medium TM and with fungal biomass of widely varying ages, no significant improvement was noted from allowing the mycelium to age. Accordingly, it was concluded that biomass for the immobilization studies should be cultured in the regular strength medium TM with harvesting occurring at the start of the stationary phase of the growth cycle.

5.3 Production of *Penicillium* Biomass for Immobilization

During the growth studies, it was noted that the start of the stationary phase of growth was associated with a sharp rise in the pH of the medium and this had been used to time the taking of samples at intervals along the stationary phase of the growth curve. However, the level of pH change varied widely between cultures *cf.* Figures 2, 4, 6, and 7. In Experiment P8, the end of the linear phase of growth cycle was marked by only a minor jump in the pH of the culture.

This inflection in the pH of the medium was used to indicate when a culture should be harvested. This protocol yielded biomasses P11 (Table 11) and P12 (Table 12) from Medium TM sterilized only once after dilution.

These biomasses were relatively young, but the loading capacities (Tables 11 and 12) matched values reported in the literature for other fungal species. Thus, uranium loadings of 260 mg/g and 320 mg/g were measured for biomasses P11 and P12, respectively.

These extreme increases in the loading capacities might appear at first glance to be correlated in part with elimination of the lag phase upon using the media sterilized in the diluted form. However, these loadings were also obtained in tests with higher liquid to solids ratios (*i.e.*, 0.02 g/400 mL mine water) than was used with most of the previous tests. Thus, the loadings obtained with 0.2 g solids/400 mL were similar to those observed with other biomass samples assessed at that solids/liquid ratio. The uncertainty in assessing small changes in the solution concentration (following biosorption), which is introduced by the uranium analysis procedure, can have a significant effect on the calculated uranium loading values for both the immobilised and unimmobilised biomass.

5.4 Assessment of Immobilized/Pelletized Biomasses

The Penicillium biomass P12, immobilized/pelletized by the Tzesos procedure, was tested for uranium adsorption as a dried product (Table 12). The Penicillium biomass P11, immobilized/pelletized by the ORF Sol Gel procedure was tested for uranium adsorption both as a wet preparation (before drying) and as the dried pellets (Table 13).

As discussed previously, the calculated loadings appear to be very sensitive to the test conditions. However, the data indicate that the immobilized and pelletized samples retain high uranium adsorption capacities in excess of 150 mg uranium/g dried biomass pellets. The adsorption isotherms from the biomass pellets appear close to linear at high liquid/solids ratios, but significant deviations occur when the ratio is lowered.

TABLE 1

MULTI ELEMENT ANALYSIS OF pH 4 MINE WATER

Element	Sample N ppm	Sample O ppm
Co	4.8	4.2
Zn	18	12
Cd	0.05	<0.05
B	0.76	0.74
Bi	<0.2	<0.2
P	<0.5	<0.5
Be	0.02	0.02
Si	25	23
Fe	3.0	1.0
Mn	2.1	2.5
Ca	140	150
Mg	16	26
Cu	2.6	1.9
Al	48	59
V	<0.02	<0.02
Mo	<0.05	<0.05
Pb	<0.1	<0.1
Ni	2.1	2.2
Cr	0.06	0.05
Na	2590	1470
U	150	153

TABLE 2

URANIUM ADSORPTION TESTS - PENICILLIUM BIOMASS - EXPERIMENT P1 (Medium TM)

Feed Solution: Mine Water, pH adj. to 4. Contact Time - 90 Hours

Test No.	Biomass	Age(1) of Biomass (h)	Biomass g	Mine Water mL	Feed Solution U ppm	Feed Solution U mg	Final Solution U ppm	Final Solution U mg	Mg U loaded	Mg U/g Biomass
87-3	P1 - 5	55	0.09	100	142	14.2	112	11.2	3.0	33.3
87-4	"	55	0.19	100	142	14.2	79	7.9	6.3	33.2
87-5	"	55	0.48	100	142	14.2	32	3.2	11.0	22.9
87-6	"	55	0.65	100	142	14.2	19	1.9	12.3	18.9

(1) Age of biomass calculated from mid-point of linear phase of growth curve.

TABLE 3

URANIUM ADSORPTION TESTS - PENICILLIUM BIOMASS - EXPERIMENT P2 - (Medium TM)

BIOMASS ADSORPTION TESTING

Feed Solution: Mine Water, pH adj. to 3.85 U = 92 ppm Contact Time - 90 Hours
Synthetic Solution pH 3.85 U =193 ppm

Test No.	Biomass	Age(1) of Biomass (h)	Biomass g	Mine Water mL	Feed Solution U ppm	Feed Solution U mg	Final Solution U ppm	Final Solution U mg	Mg U loaded	Mg U/g Biomass
87-8	P2 - 12 Autoclaved	159	0.1	100	92	9.2	13	1.3	7.9	79
87-9	"	159	0.2	100	92	9.2	5.1	0.51	8.69	43.4
87-10	"	159	0.5	100	92	9.2	0.9	0.09	9.11	18.2
87-11	"	159	0.1	100	193	19.3	7.2	0.72	18.58	186
87-12	"	159	0.2	100	193	19.3	2.7	0.27	19.03	95
87-13	"	159	0.5	100	193	19.3	0.8	0.08	19.22	38.4

(1) See footnote - Table 2

TABLE 4

URANIUM ADSORPTION TESTS - PENICILLIUM BIOMASS - EXPERIMENT P2 (Medium TM)

Feed Solution: Mine Water, pH adj. to 4.0 Contact Time - 24 Hours

Test No.	Biomass	Age(1) of Biomass (h)	Biomass g	Mine Water mL	Feed Solution U ppm	Feed Solution U mg	Final Solution U ppm	Final Solution U mg	Mg U loaded	Mg U/g Biomass
87-15	P2-8 Autoclaved	42.5	0.1	100	89	8.9	44	4.4	4.5	45
87-16	"	42.5	0.2	100	89	8.9	31	3.1	5.8	29
87-17	"	42.5	0.5	100	89	8.9	7.1	0.71	8.2	16.4
87-18	P2-11 Autoclaved	105	0.1	100	89	8.9	28	2.8	6.1	61
87-19	"	105	0.2	100	89	8.9	5.3	0.53	8.4	42
87-20	"	105	0.5	100	89	8.9	tr	-	8.92	17.8
87-21	P2-12 Inactivated 75°C Ethanol	159	0.1	100	89	8.9	4.4	0.44	8.5	85
87-22	"	159	0.2	100	89	8.9	tr	-	8.9	44.5
87-23	"	159	0.5	100	89	8.9	tr	-	8.9	17.8
87-24	P2-12 Inactivated 85°C	159	0.1	100	89	8.9	5.6	0.56	8.3	83
87-25	"	159	0.2	100	89	8.9	tr	-	8.9	44.5
87-26	"	159	0.5	100	89	8.9	tr	-	8.9	17.8

(1) See footnote - Table 2.

TABLE 5

URANIUM ADSORPTION TESTS - PENICILLIUM BIOMASS - EXPERIMENTS P3 (Medium TM) P4 (Medium TM) and PE6 (Alternative Carbon Sources)

Feed Solution: Mine Water, pH adj. to 4.05 Contact Time - 24 Hours

Test No.	Biomass	Age(1) of Biomass (h)	Biomass g	Mine Water mL	Feed Solution U ppm	Feed Solution U mg	Final Solution U ppm	Final Solution U mg	Mg U loaded	Mg U/g Biomass
87-32	P3-13	210	0.1	200	66	13.2	37	7.4	5.8	58
87-33	P4-11	270	0.1	200	66	13.2	21	4.2	9.0	90
87-34	PE6-1 (2)	~70	0.1	200	66	13.2	42	8.4	4.8	48
87-35	PE6-2 (3)	~40	0.1	200	66	13.2	38	7.6	5.6	56
87-36	PE6-3 (4)	~40	0.1	200	66	13.2	43	8.6	4.6	46

(1) See footnote - Table 2.
(2) Carbon source - potato starch
(3) Carbon source - lactose
(4) Carbon source - maltodextrins

TABLE 6

URANIUM ADSORPTION TESTS - PENICILLIUM BIOMASS - EXPERIMENT P2 (Medium TM)
NEW SAMPLE OF MINE WATER

Feed Solution: Mine Water, pH adj. to 4.0 Contact Time - 24 Hours

Test No.	Biomass	Age(1) of Biomass (h)	Biomass g	Mine Water mL	Feed Solution U ppm	Feed Solution U mg	Final Solution U ppm	Final Solution U mg	Mg U loaded	Mg U/g Biomass
87-40	P2-12	159	0	200	135	27	135	27	0	0
87-41	"	159	0.01	200	135	27	126	25.2	1.8	180
87-42	"	159	0.02	200	135	27	129	25.8	1.2	60
87-43	"	159	0.03	200	135	27	124	24.8	2.2	73.3
87-44	"	159	0.05	200	135	27	115	23.0	4.9	80
87-45	"	159	0.1	200	135	27	98	19.6	7.42	74

(1) See footnote - Table 2.

TABLE 7

URANIUM ADSORPTION TESTS - PENICILLIUM BIOMASS GROWN ON VARIOUS CARBON SOURCES

Feed Solution: Mine Water, pH adj. to 4.0 Contact Time - 24 Hours

Test No.	Biomass	Age(1) of Biomass (h)	Biomass g	Mine Water mL	Feed Solution U ppm	Feed Solution U mg	Final Solution U ppm	Final Solution U mg	Mg U loaded	Mg U/g Biomass
87-51	P2-12 (2)	159	0.014	200	120	24	111	22.2	1.8	129
87-52	P3-13 (3)	210	0.01	200	120	24	112	22.4	1.6	160
87-61	P4-11 (4)	270	0.1	200	51	10.2	6.3	1.3	8.9	89
87-62	P5 (5)	n/a	0.1	200	51	10.2	39	7.8	2.4	24
87-63	P6 (6)	200	0.1	200	51	10.2	13	2.6	7.6	76
87-64	PE7-1 (7)	n/a	0.1	200	51	10.2	18	3.6	6.6	66
87-65	PE7-2 (8)	n/a	0.1	200	51	10.2	30	6.0	4.2	42

(1) See footnote - Table 2.
(2) Medium TM
(3) Medium TM
(4) Medium TM/4
(5) Carbon source - hexadecane
(6) Carbon source - cheese whey
(7) Carbon source - potato starch
(8) Carbon source - lactose

TABLE 8

URANIUM ADSORPTION TESTS - PENICILLIUM BIOMASS - EXPERIMENTS P8 (Medium TM/2) AND P9 (Medium TM/1.5)

Feed Solution: Mine Water, pH adj. to 4.0 Contact Time - 24 Hours

Test No.	Biomass	Age(1) of Biomass (h)	Biomass g	Mine Water mL	Feed Solution U ppm	Feed Solution U mg	Final Solution U ppm	Final Solution U mg	Mg U loaded	Mg U/g Biomass
87-66	P8-12A	129	0.1	200	122	24.4	109	21.8	2.6	26
87-67	P8-12B	129	0.1	200	122	24.4	116	23.2	1.2	12
87-68	P8-15A	239	0.1	200	122	24.4	112	22.4	2.0	20
87-69	P8-15B	239	0.1	200	122	24.4	117	23.4	1.0	10
87-70	" -Final	385	0.1	200	122	24.4	121	24.2	0.2	2
87-71	P9-11A	85	0.1	200	122	24.4	117	23.4	1.0	10
87-72	P9-11B	85	0.1	200	122	24.4	120	24.0	0.4	4
87-73	" -13A	180	0.1	200	122	24.4	112	22.4	2.0	20
87-74	" -13B	180	0.1	200	122	24.4	+122		Erratic	Analysis
87-75	P9 Final	327	0.1	200	122	24.4	+122		"	"

(1) See footnote - Table 2.

TABLE 9

URANIUM ADSORPTION TESTS - PENICILLIUM BIOMASS - EXPERIMENT P8 (Medium TM/2) AND P9 (Medium TM/1.5)

Feed Solution: Mine Water, pH adj. to 4.0 Contact Time - 24 Hours

Test No.	Biomass	Age(1) of Biomass (h)	Biomass g	Mine Water mL	Feed Solution U ppm	Feed Solution U mg	Final Solution U ppm	Final Solution U mg	Mg U loaded	Mg U/g Biomass
87-76	P8-12A	129	0.1	200	150	30	131	26.2	3.8	38
87-77	"	129	0.1	400	150	60	133	53.2	6.8	68
87-78	"	129	0.1	200	153	30.6	125	25.0	5.6	56
87-79	"	129	0.1	400	153	61.2	133	53.2	8.0	80
87-80	"	129	0.1	200	150	30	130	26.0	4.0	40
87-81	P8-15A	239	0.1	200	150	30	124	24.8	5.2	52
87-82	P9-11A	85	0.1	200	150	30	130	26.0	4.0	40
87-83	P9-13A	180	0.1	200	150	30	122	24.4	5.6	56
87-84	P8-Final	1385	0.1	200	177	35.4	171	34.2	1.2	12
87-85	P9-Final	1327	0.1	200	177	35.4	167	33.4	2.0	20

(1) See footnote - Table 2.

TABLE 10

URANIUM ADSORPTION TESTS - PENICILLIUM BIOMASS - EXPERIMENT P10 (Medium TM)

Feed Solution: Mine Water, pH adj. to 4.0 Contact Time - 24 Hours

Test No.	Biomass	Age(1) of Biomass (h)	Biomass g	Mine Water mL	Feed Solution U ppm	Feed Solution U mg	Final Solution U ppm	Final Solution U mg	Mg U loaded	Mg U/g Biomass
87-86	P10-11	5	0.1	200	148	29.6	125	25.0	4.6	46
87-87	P10-12	31	0.1	200	148	29.6	124	24.8	4.8	48
87-88	P10-11	5	0.1	200	142	28.4	120	24.0	4.4	44
87-89	P10-12	31	0.1	200	142	28.4	120	24.0	4.4	44
87-90	P10-14	79	0.1	200	120	24.0	100	20.0	4.0	40
87-91	P10-17	154	0.1	200	120	24.0	95	19.0	5.0	50
87-92	P10-15	106	0.1	200	120	24.0	96	19.2	4.8	48

(1) See footnote - Table 2.

TABLE 11

URANIUM ADSORPTION TESTS - PENICILLIUM BIOMASS - EXPERIMENT P11 (Medium TM)

Feed Solution: Mine Water, pH adj. to 4.0 Contact Time - 24 Hours

Test No.	Biomass	Age(1) of Biomass (h)	Biomass g	Mine Water mL	Feed Solution U ppm	Feed Solution U mg	Final Solution U ppm	Final Solution U mg	Mg U loaded	Mg U/g Biomass
87-93	P11-6	16.5	0.02	400	153	61.2	140	56.0	5.2	260
87-94	"	16.5	0.02	400	153	61.2	140	56.0	5.2	260
87-95	"	16.5	0.04	400	153	61.2	140	56.0	5.2	130
87-96	"	16.5	0.06	400	153	61.2	140	56.0	5.2	86.7
87-97	"	16.5	0.1	400	162	64.8	151	60.4	4.4	44
87-98	"	16.5	0.1	400	162	64.8	149	59.6	5.2	52
87-99	"	16.5	0.2	400	162	64.8	148	59.2	5.6	28
87-100	"	16.5	0.2	400	162	64.8	148	59.2	5.6	28

(1) See footnote - Table 2.

TABLE 12

URANIUM ADSORPTION TESTS - PENICILLIUM BIOMASS P12
IMMOBILIZED/PELLETIZED BY TSEZOS PROCEDURE

Feed Solution: Mine Water, pH adj. to 4.05 Contact Time - 168 Hours

Test No.	Pelletized Biomass g	Mine Water mL	Feed Solution U ppm	Feed Solution U mg	Final Solution U ppm	Final Solution U mg	Mg U loaded	Mg U/g Pellets
87-101	0.02	400	166	66.4	150	60.0	6.4	320
87-102	0.04	400	166	66.4	150	60.0	6.4	160
87-103	0.06	400	166	66.4	149	59.6	6.8	113
87-104	0.1	400	166	66.4	148	59.2	7.2	72
87-105	0.2	400	166	66.4	143	57.2	9.2	26

(1) See footnote - Table 2.

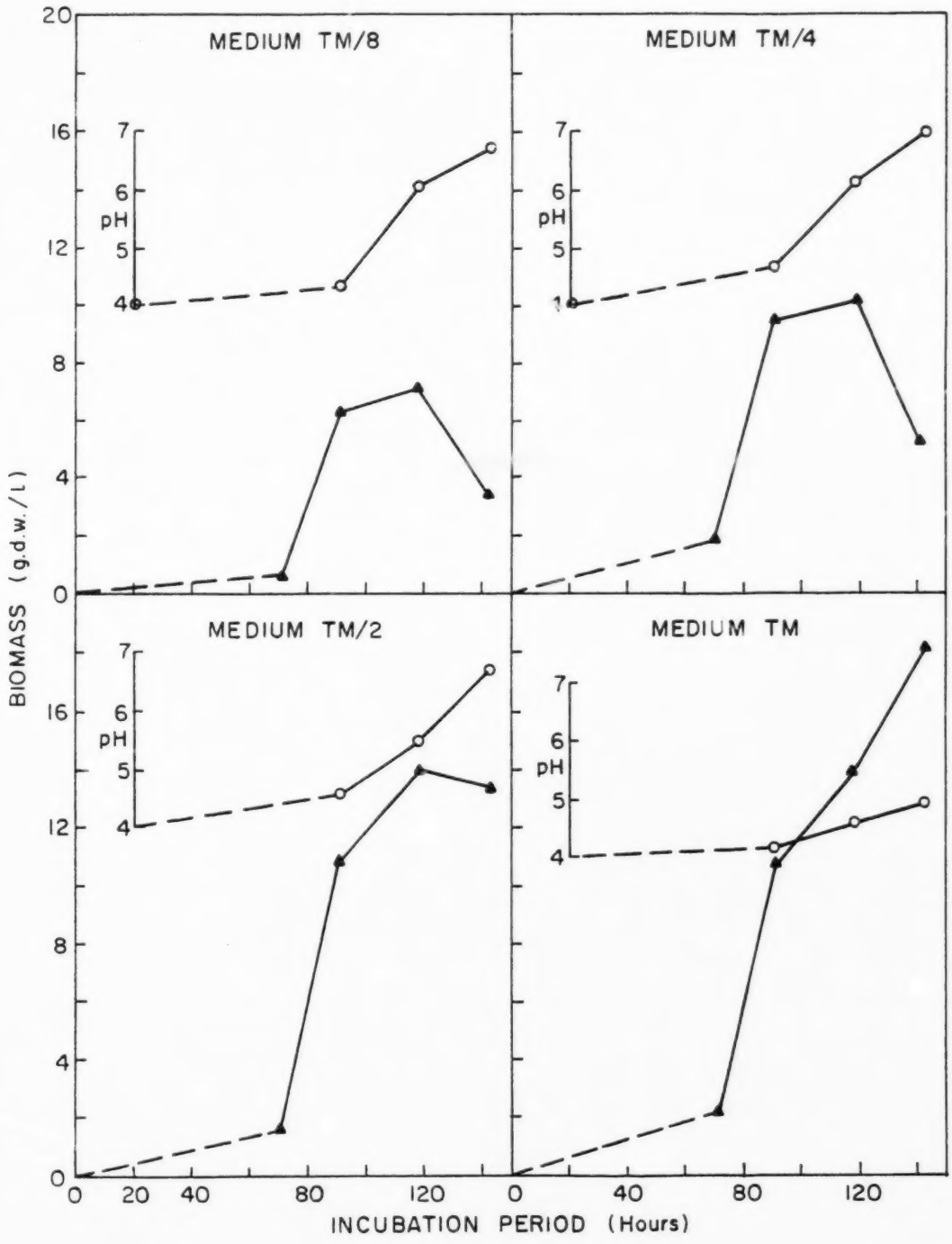
TABLE 13

URANIUM ADSORPTION TESTS - PENICILLIUM BIOMASS P11 IMMOBILIZED/ PELLETIZED BY ORF SOL GEL PROCEDURE

Feed Solution: Mine Water, pH adj. to 4.0 Contact Time - 24 Hours

Test No.	Biomass	Biomass g	Mine Water mL	Feed Solution U ppm	Feed Solution U mg	Final Solution U ppm	Final Solution U mg	Mg U loaded	Mg U/g Pellets
87-106	Wet	0.02	400	166	66.4	151	60.4	6.0	300
87-107	"	0.1	400	166	66.4	142	56.8	9.6	96
87-108	"	0.2	400	166	66.4	119	47.6	18.8	94
87-109	Dry	0.02	400	166	66.4	146	58.4	8.0	400
87-110	"	0.02	400	166	66.4	142	56.8	9.6	480
87-111	"	0.04	400	166	66.4	142	56.8	9.6	240
87-112	"	0.06	400	166	66.4	141	56.4	10.0	167
87-113	"	0.1	400	166	66.4	137	54.8	11.6	116
87-114	"	0.2	400	166	66.4	133	53.2	13.2	66

FIGURE 1 SHAKE FLASK CULTURES - MEDIUM TM



KEY: ▲ BIOMASS (g.d.w./l)
○ pH

FIGURE 2 GROWTH CURVE OF PENICILLIUM SP. - MEDIUM TM
CULTURE P2

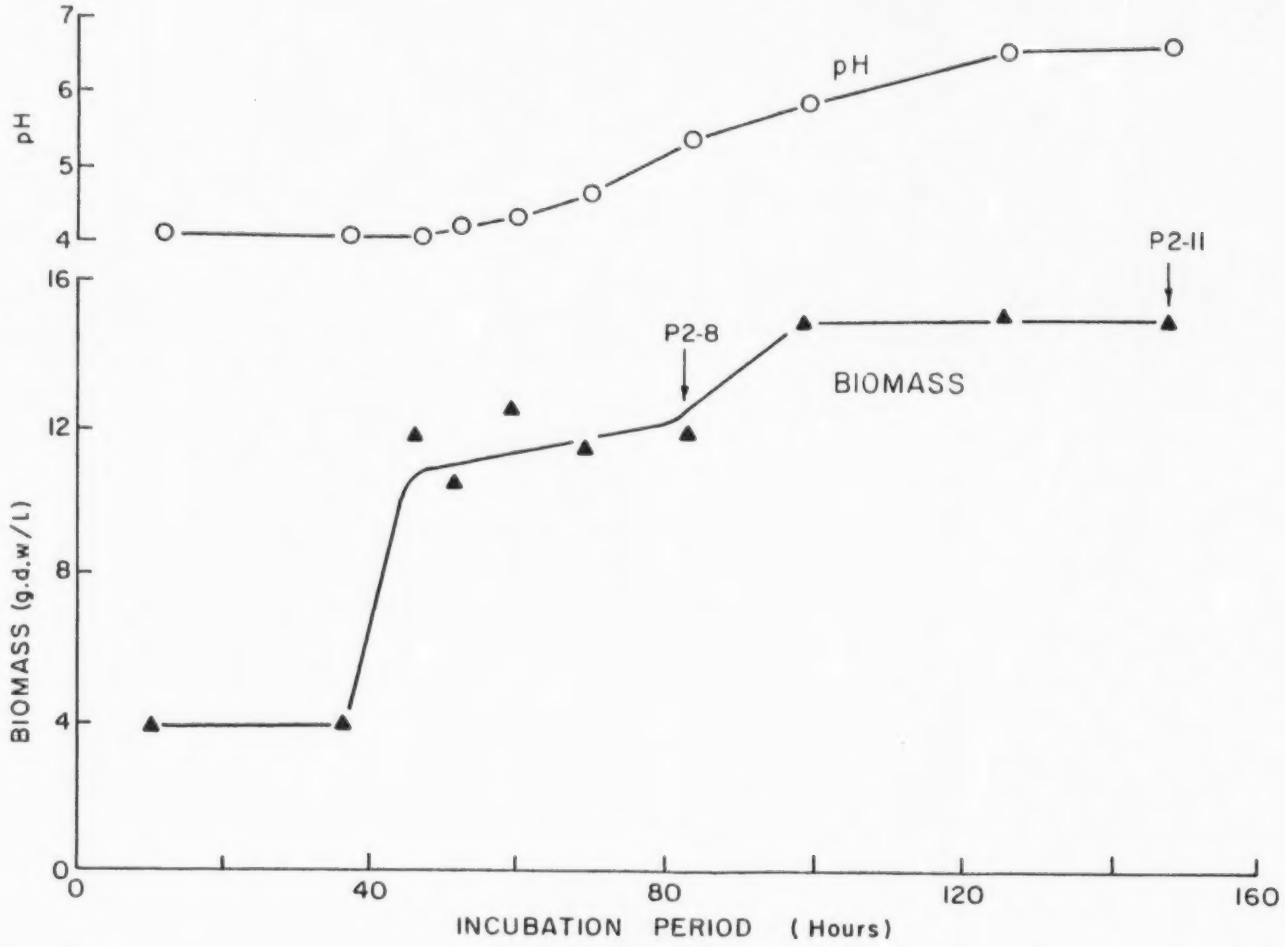


FIGURE 3 GROWTH CURVE OF PENICILLIUM SP. - MEDIUM TM CULTURE P3

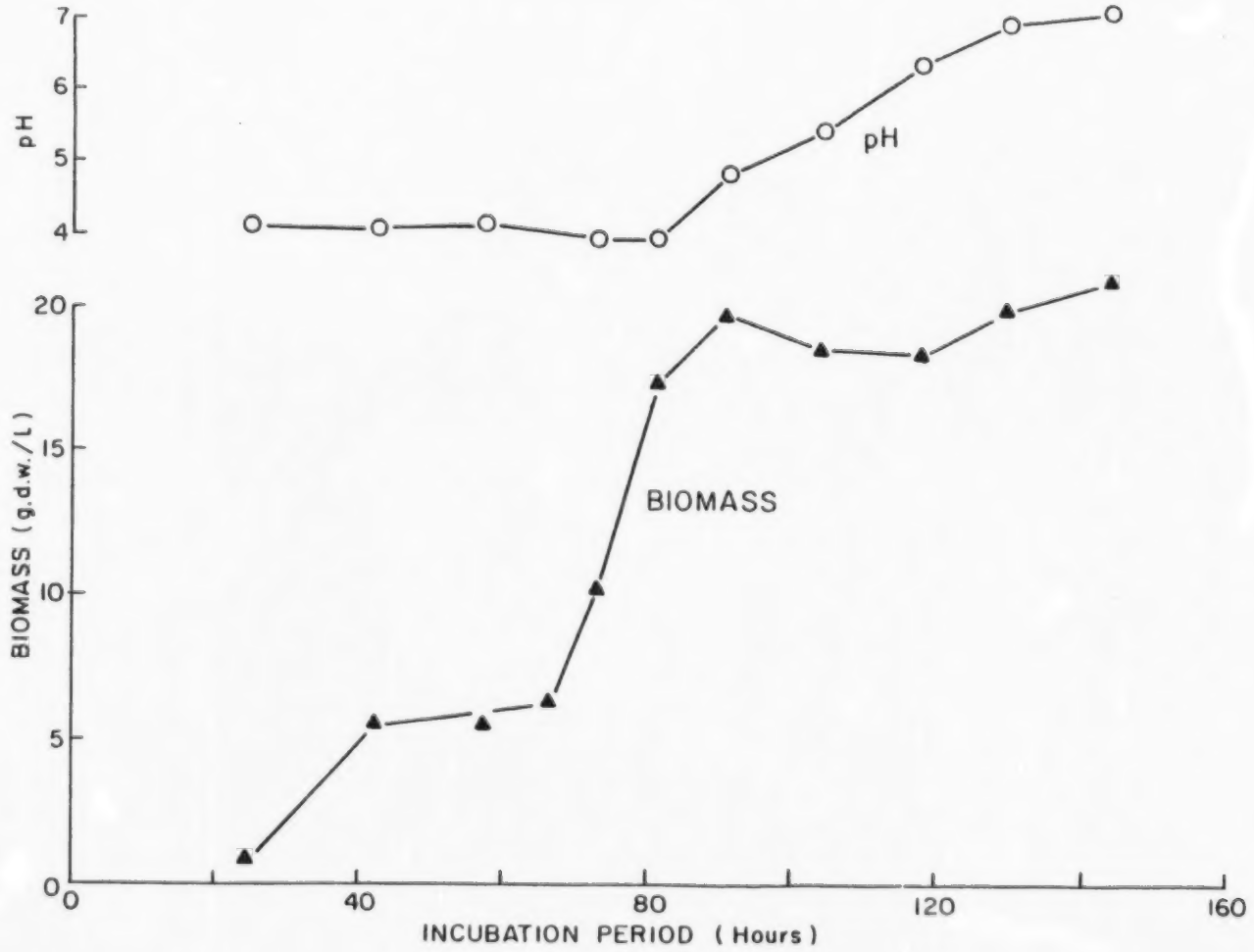


FIGURE 4 GROWTH CURVE OF PENICILLIUM SP. - MEDIUM TM/4
CULTURE P4

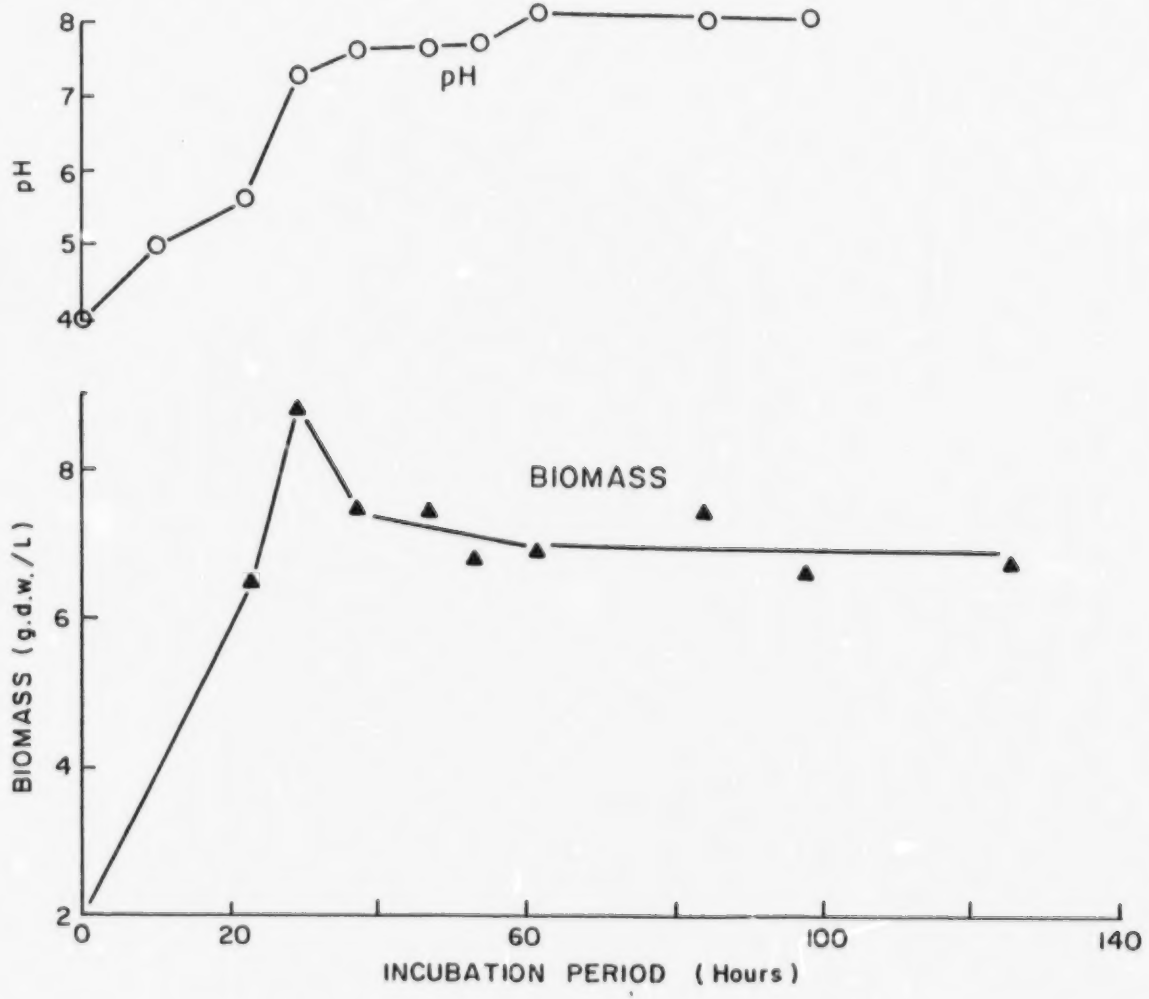


FIGURE 5 GROWTH CURVE OF PENICILLIUM SP. IN CHEESE WHEY CULTURE P6

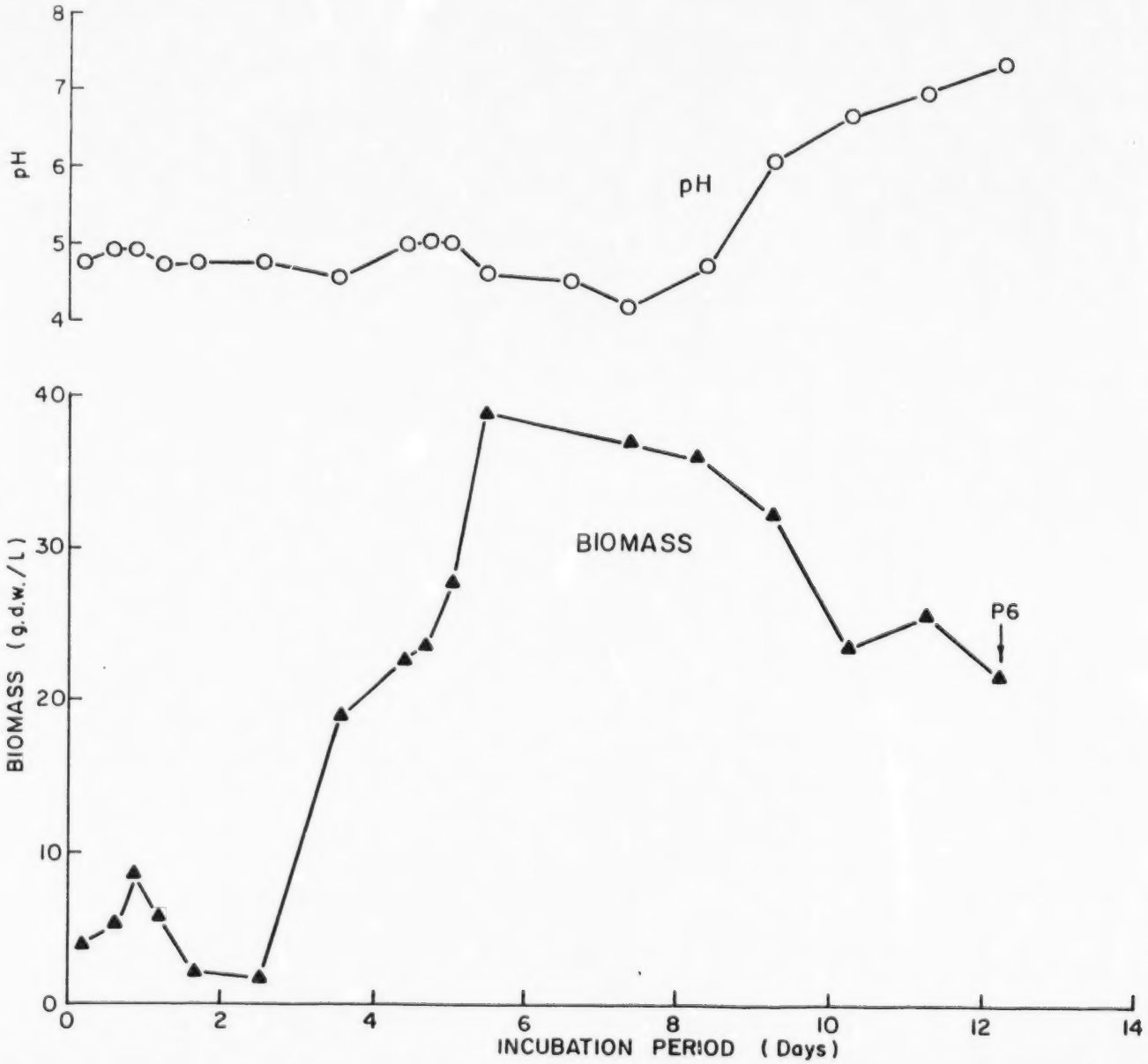


FIGURE 6 GROWTH CURVE OF PENICILLIUM SP. - MEDIUM TM/2
CULTURE P8·

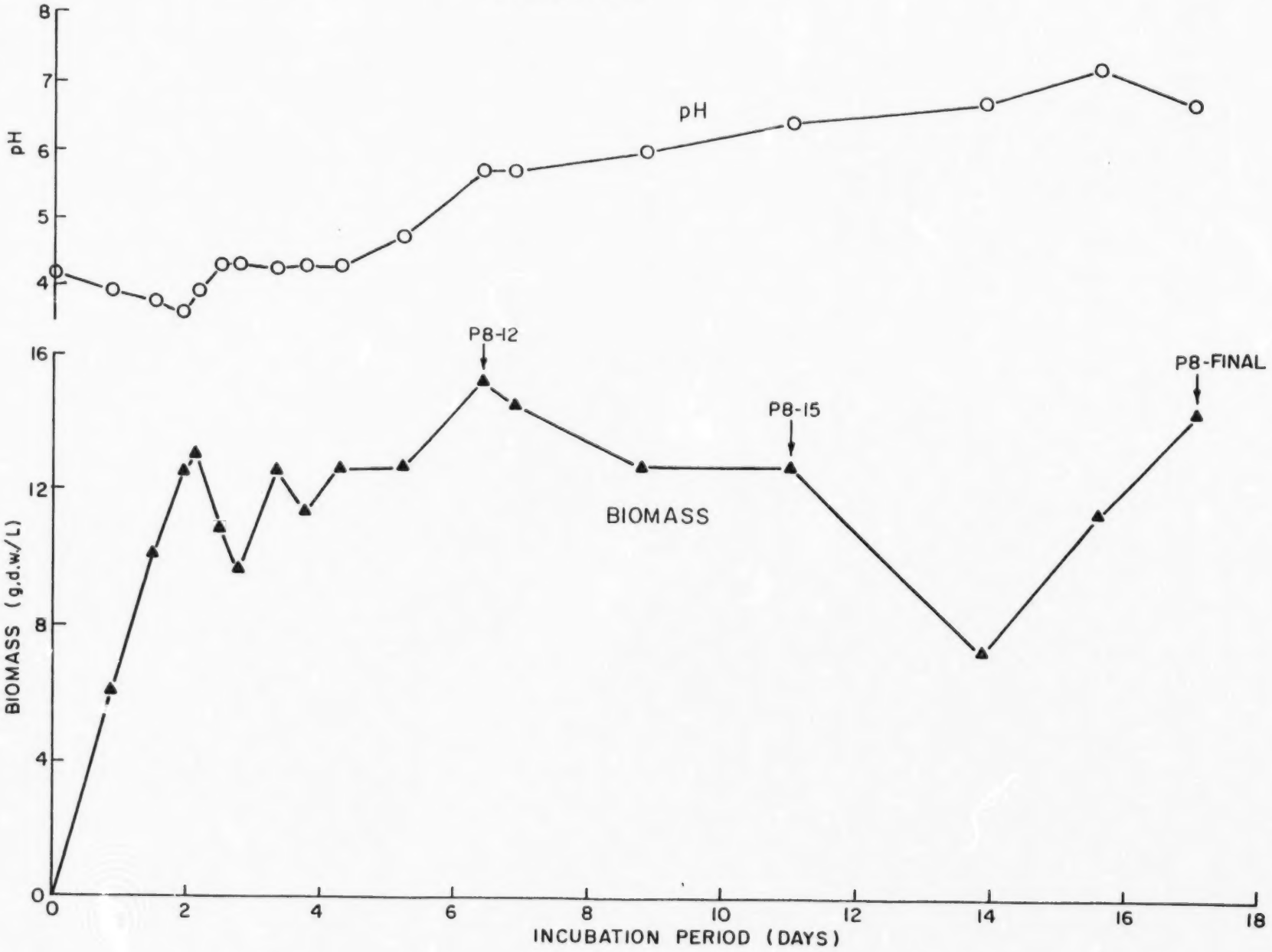


FIGURE 7 GROWTH CURVE OF PENICILLIUM SP. - MEDIUM TM/1.5
CULTURE P9

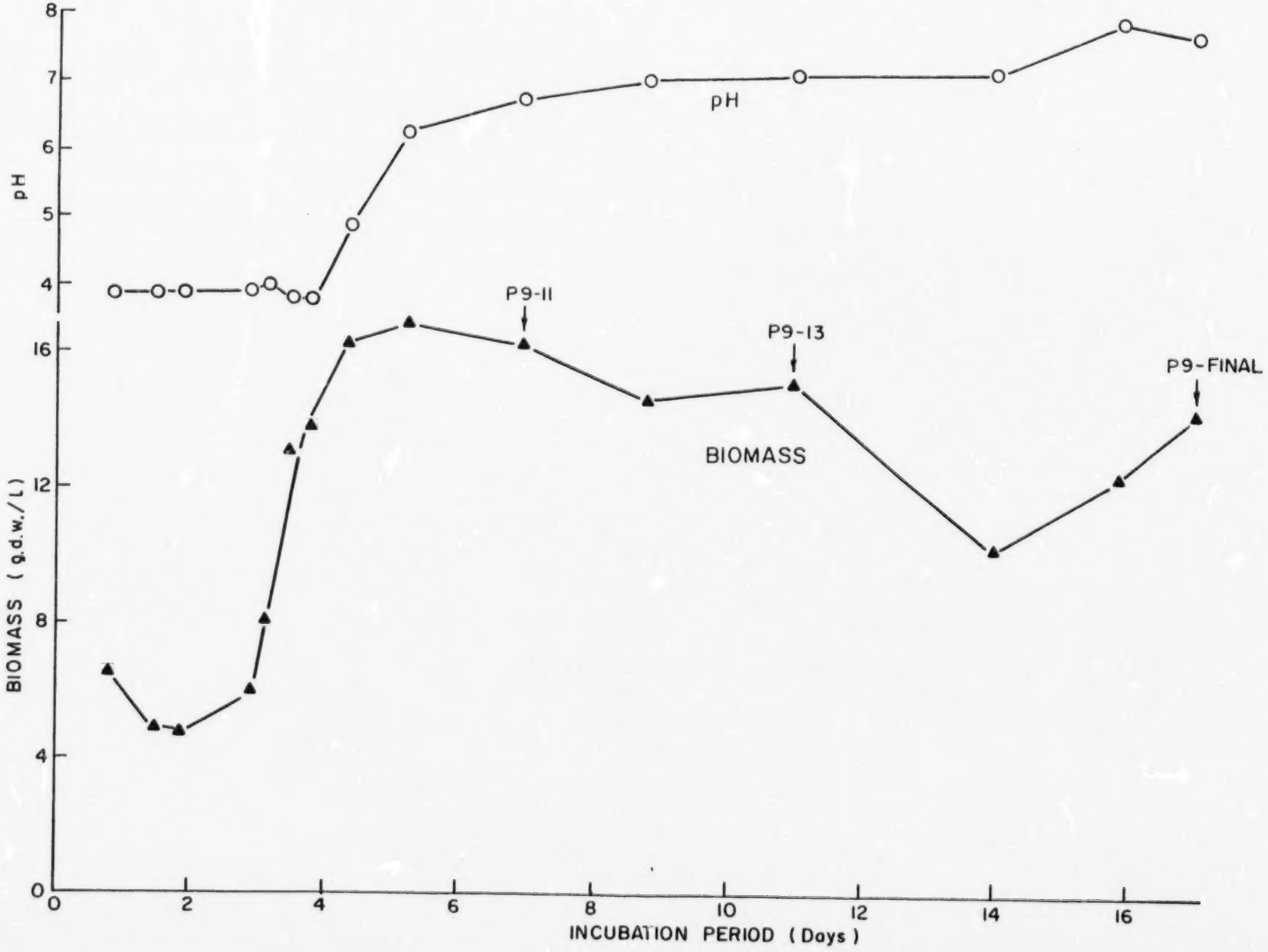


FIGURE 8 GROWTH CURVE OF PENICILLIUM SP. IN MEDIUM TM CULTURE P10

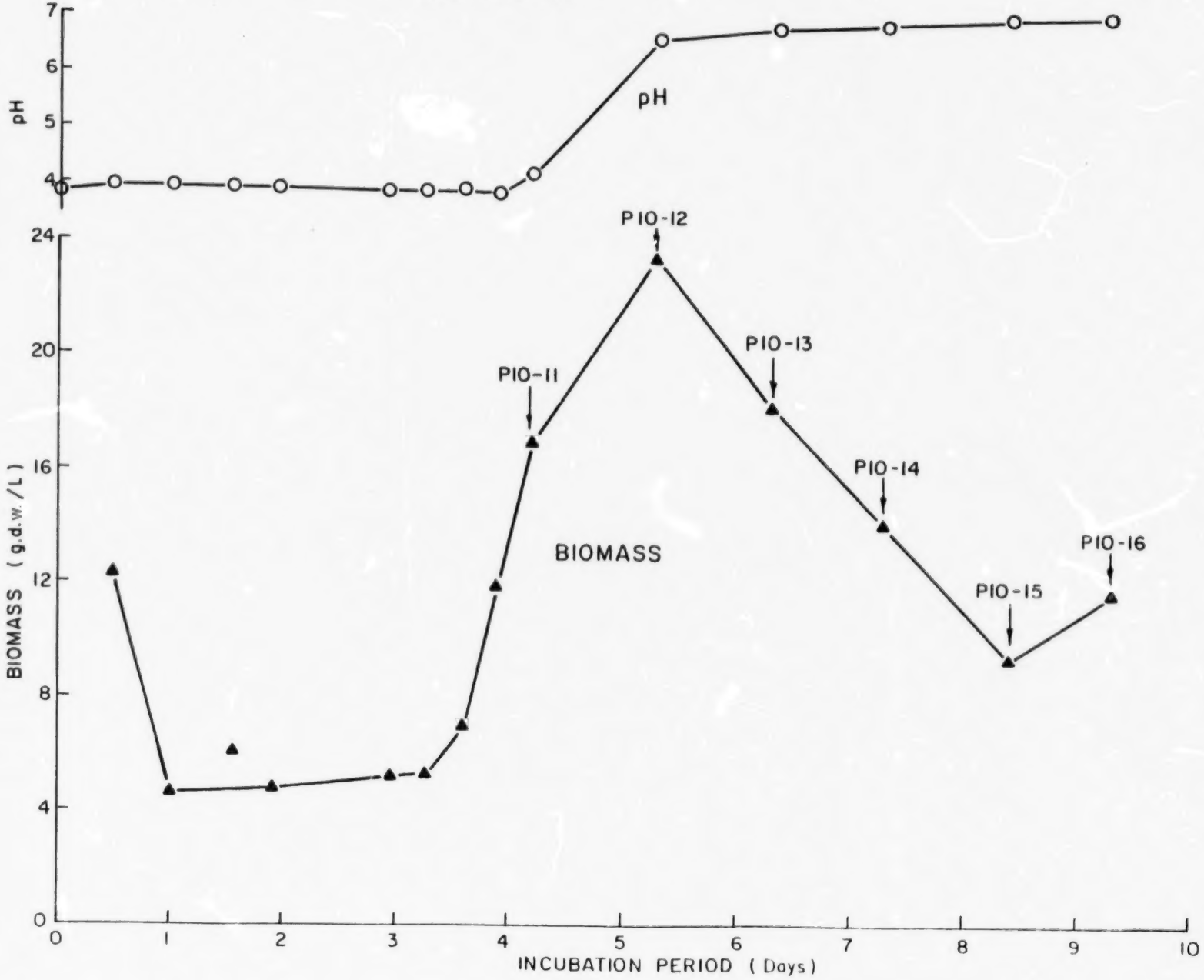


FIG. 9 GROWTH CURVE OF PENICILLIUM SP IN MEDIUM TM - LARGE SCALE PREPARATION OF BIOMASS. CULTURE PII

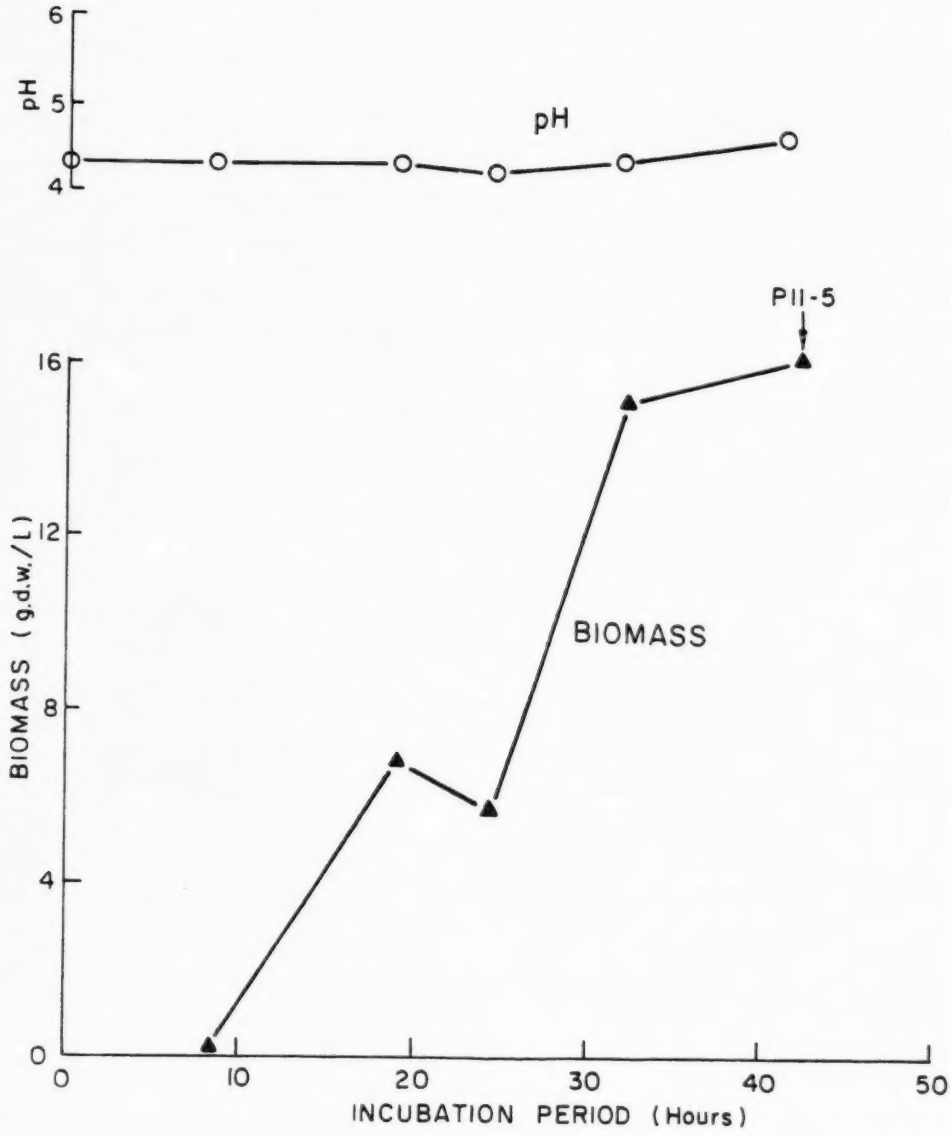


FIG. 10 GROWTH CURVE OF PENICILLIUM SP. IN MEDIUM TM - LARGE SCALE PRODUCTION OF BIOMASS

pH CURVE CULTURE P12

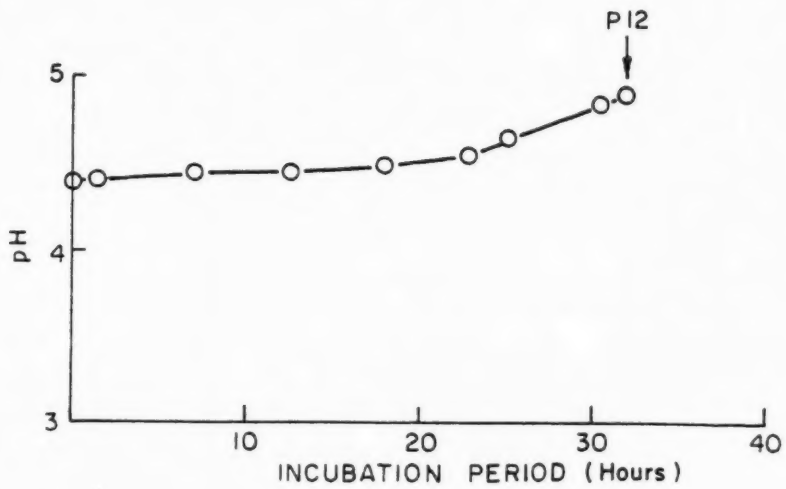


FIGURE II GROWTH CURVE OF PENICILLIUM SP. IN MEDIUM TM STERILIZED AFTER DILUTION OF CONCENTRATE CULTURE P13

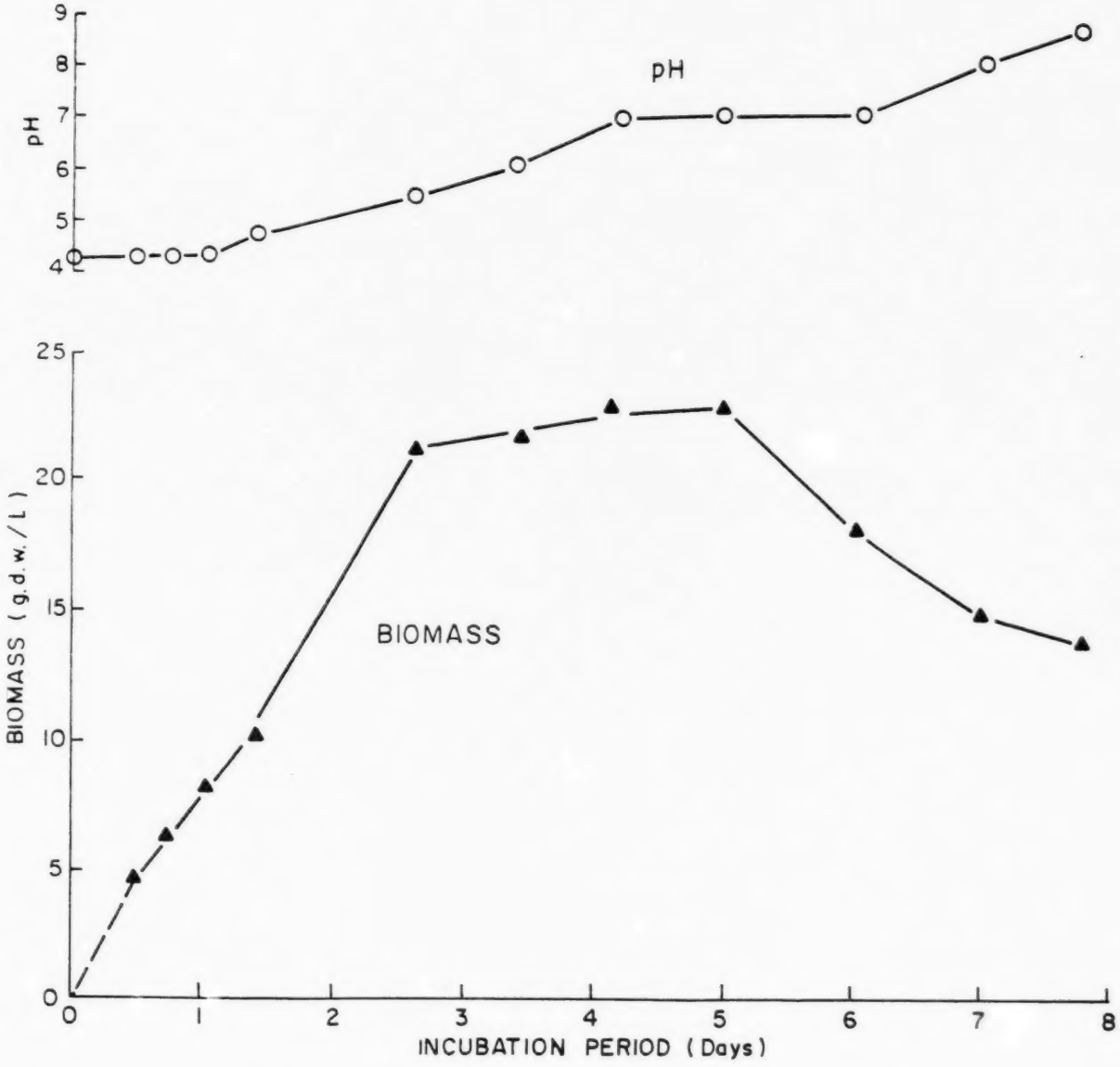


FIGURE 12 COMPARISON OF STERILIZATION PROTOCOL ON GROWTH OF PENICILLIUM SP. IN MEDIUM TM